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THE RELATIVE PROPORTION OF BACTERIA IN TOP MILK (CREAM LAYER) AND BOTTOM MILK (SKIM MILK), AND ITS BEARING ON INFANT FEEDING.*†

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IN the course of a study on tubercle bacilli in market milk[1] and of a later study on the best procedure for their detection in milk it was noticed that when guinea-pigs were inoculated with the cream a very much higher percentage died from acute infections than when the sediment was used. The inference was natural that the cream contained more bacteria than the bottom milk or sediment. A few preliminary examinations having shown this supposition to be correct, a study was begun as to the number of bacteria in the whole milk, the bottom milk or sediment, and the cream, both that collected by gravity and by centrifugation.

The relative number of bacteria in the top milk and in the bottom milk is a subject of very great importance in the modification of milk for infant-feeding. All the writers on pediatrics and infant-feeding give formulae for the modification of milk based upon the use of various amounts of top milk. My studies show that top milk, such as is advised for use in the above formulae, contains from 10 to 500 times as many bacteria per c.c. as the mixed milk. This preponderance of bacteria in top milk may account for the fact that sometimes children do not thrive on modified milk when made from top milk, but improve when the whole milk is used for modification.

The various bacteria causing acute infections, as well as tubercle bacilli, are more numerous in the top milk than in the bottom milk. In many cases this difference is more than a hundred fold and, as infection must depend to some extent on the number of bacteria intro-

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[1] Anderson, John F., "The Frequency of Tubercle Bacilli in the Market Milk of the City of Washington, D. C." *Bull. No. 41, Hyg. Lab., U. S. Pub. Health and Mar. Hosp Serv.*, 1908, p. 163.

duced into the body, too little attention has been given to the question of the number of bacteria in top milk when used for infant-feeding. Oftentimes when infants are taken off breast milk and put on modified cow's milk made from top milk it is found that, in spite of various modifications containing varying percentages of proteid, the milk fails to agree with the infant. In those cases which develop diarrhea the fault may not always be in the proteids, but in the large number of bacteria in the top milk used for the preparation of the formula.

The greater frequency of intestinal tuberculosis in young children may be due, not only to the fact that they use a large amount of milk, but because top milk, which contains more tubercle bacilli per c.c. than the whole milk, is used in the preparation of modified-milk formulae.

The literature upon the subject of the relative number of bacteria in top milk and in bottom milk is very slight. None of the writers seems to have realized the great importance of the subject in its relation to infant feeding.

Wyss[1] investigated the question whether, by centrifugation, milk is freed of a part of its bacteria or whether the organic substance of the milk sediment does not, for the greater part, consist of bacteria. His work resulted in the determination that the precipitate was seven times richer in bacteria than the centrifugalized milk.

Scheurlen[2] found in his experiments with pathogenic bacteria other than the tubercle bacilli in centrifugalized milk a marked bacterial increase in the sediment and the cream, and a marked decrease in the bottom milk. Among others, he gives the following example of the distribution of bacteria by centrifugalization of milk purchased from the Bolle dairy in Berlin:

Milk contained	2,050,000	bacteria	per c.c.
Cream	8,500,000	"	"
Bottom milk	700,000	"	"
Milk dirt	18,000,000	"	in 0.6 c.c.

Niederstadt[3] draws the conclusion that in the centrifugalization of milk 75 per cent of the bacteria go to the cream, while the rest remain in the bottom milk. Centrifugalization does not free the milk of its bacteria.

Wilkens[4] found that the greater part of the bacteria go into the cream on centrifugalizing and that the total number was reduced by the process, not alone through the removal of the sediment; he supposed that some of them were killed.

Russell[5] states that cream, whether secured by gravity or by a cream separator, is invariably richer in bacteria than the skimmed milk of the same age. A sample of milk might have less than 100,000 bacteria per c.c. in the skimmed part, while the number in the cream layer would be several millions. Gravity raised cream is usually richer in bacteria than separator cream, mainly because it is older. He cites Poff and Becker as saying that a sample of milk which, before it was separated, had 73,000 bacteria per c.c., had the following after separation:

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[1] *Centralbl. f. Bakt.*, 1889, 6, p. 587.

[2] *Arb. a. d. kais. Gesundhtsamte*, 1891, 7, pp. 269–82.

[3] *Kochs Jahresber. u. d. Fortschr. in der Lehre v. d. Gärungs-Organismen*, 1893, 4, p. 205.

[4] *Centralbl. f. Bakt., Ref.*, 1894, 16.

[5] *Outline of Dairy Bacteriology*, Madison, 1894, pp. 127.

Cream	58,275
Skimmed milk	21,500
Slime	43,900

Backhaus and Cronheim[1] did not find that the cream obtained by centrifugalization was much richer than the bottom milk in bacteria. They obtained the following results with two samples of milk.

Whole milk	1,910,000	bacteria per c.c.
	3,420,000	" "
Cream	1,740,000	" "
	3,110,000	" "
Bottom milk	1,450,000	" "
	2,600,000	" "

Severin[2] states that, under the influence of centrifugalization, the milk is separated into three layers: The dirt particles, being the heaviest of the ingredients, are thrown to the periphery where they remain clinging to the walls of the centrifuge; then comes the whey ring; while in the central portion of the centrifuge is the cream, which is the lightest part of the milk. The bacteria of the milk are also influenced by the centrifugalization. Organisms with higher specific gravity than the milk are thrown to the periphery; those with lower are removed to the center. This peculiar distribution of the bacteria in the milk is manifestly complicated by the question whether the organisms possess specific movement or not.

Hess[3] found, in his study on the distribution of bacteria in bottled milk, that the bacteria are much more numerous in the upper layers of the cream, gradually becoming fewer in the lower portion of the bottle. The upper two ounces contain the greatest number of bacteria. He found this to be true of the tubercle bacilli, as well as the staphylococci and other bacteria.

METHODS.

Pint bottles of milk were purchased from different dairies each day and at once brought to the laboratory. As soon as received the milk was packed in cracked ice in order to allow the cream to separate as well as possible; two hours was usually sufficient for this purpose, though it was found that some bottles failed to show a distinct creamline in that time. An effort was made to avoid purchasing the milk from the same dairy more than twice.

The following dilutions were, with a few exceptions, used throughout the work: 1 c.c. of the milk +99 c.c. sterile water; 0.1 c.c., which represented a dilution of 1:1,000, was used for the first plate. One c.c. of the first dilution was added to 99 c.c. sterile water; 1 c.c. of this dilution represented 0.0001 c.c. of the original milk, and 0.1 c.c. represented 0.00001 c.c. Three plates were made from each sample. The mixtures were vigorously shaken in order to break up the clumps of bacteria and obtain as uniform a suspension of bacteria as possible. The amount of each dilution to be plated was measured directly into a petri dish and melted agar poured in the plate. The plates were kept at 37° C. for 24 hours and the colonies then counted.

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[1] *Kochs Jahresber. u. d. Fortschr. in der Lehre v. d. Gärungs-Organismen*, 1897, 8, p. 150.

[2] *Centralbl. f. Bakt.*, Abt. 2, 1905, 14, p. 605.

[3] *Pediatrics*, New York, August, 1908.

THE RELATIVE NUMBER OF BACTERIA IN THE CREAM LAYER AND IN THE SEDIMENT LAYER OF BOTTLE MILK.

In the first series of comparative counts 35 samples of milk were studied. From each sample counts were made of the number of bacteria in the cream layer and in the sediment layer.

TABLE 1.

NUMBER OF BACTERIA IN THE GRAVITY CREAM AND SEDIMENT LAYERS.

Sample	Bacteria in Cream Layer	Bacteria in Sediment Layer
1A	405,000	2,000
2A	4,500,000	83,000
2B	1,728,000	77,000
2C	16,200,000	1,674,000
3A*	114,000,000	106,800,000
3B	62,100,000	2,600,000
3C*	637,200,000	456,000,000
4A	367,200,000	12,000,000
4B	46,800,000	2,200,000
4C	194,400,000	22,200,000
5A	145,200,000	1,500,000
5B	396,000,000	1,640,000
5C	82,400,000	2,760,000
6A	70,800,000	9,300,000
6B	67,200,000	1,200,000
6C	16,680,000	1,055,000
7A*	300,000	200,000
7B	800,000	30,000
7C	6,200,000	290,000
8A	10,500,000	1,200,000
8B*	360,000	280,000
8C	7,200,000	260,000
8D	210,000	40,000
8E	140,000	10,000
8F	140,000	
8G	1,900,000	200,000
8H	80,000	20,000
8I*	120,000	100,000
8J	870,000	70,000
9A	1,800,000	312,000
9B	160,800,000	6,360,000
9C	7,080,000	800,000
10A	6,200,000	240,000
10B	248,400,000	11,200,000
10C	152,400,000	51,600,000
Average	69,211,000	4,360,000

* Cream not separated after standing two hours; excluded from calculation of the averages.

For the cream counts the point of the pipette was put just below the surface and 1 c.c. drawn into it and used for the dilutions.

For the sediment-layer counts the pipette was plunged into the bottom of the bottle, 1 c.c. drawn up and then diluted.

It was noted that in those milks which did not show a good cream-line in two hours or which contained much dirt, the difference in the number of bacteria in the cream and sediment layers was not as marked as in those which had a distinct cream-line or were fairly free from dirt.

As will be seen from Table 1, the average number of bacteria in the cream layer of the 30 samples used in the calculation of the averages was 69,211,000. The average number in the sediment layer of the same sample was 4,360,000; or, there were about 16 times as many bacteria in the cream layer as in the sediment layer.

RELATIVE NUMBER OF BACTERIA IN GRAVITY CREAM, CENTRIFUGAL CREAM, AND IN THE SEDIMENT LAYER.

In studying the relative number of bacteria in gravity and centrifugally raised cream and in the sediment layers, the same sample of milk was used. After planting from the cream and sediment layers of the bottle of market milk, as in the preceding series, the milk was thoroughly mixed and 200 c.c. placed in a centrifuge flask. It was centrifugalized one hour at about 1,500 revolutions per minute. The cream was carefully removed and 1 c.c. of it was diluted and plates made in the usual way. After the cream had been removed 1 c.c. of the sediment was diluted and plated.

It will be seen from Table 2 that the average number of bacteria in the 26 samples of gravity cream was 68,690,000 and in the sediment 4,840,000; that is, about 14 times as many bacteria were in the cream as in the sediment.

When we come to compare the number of bacteria in the centrifugalized cream and sediment we find that the average in the cream was 97,690,000, and in the sediment 18,840,000; or only about 5.1 times as many in the cream as in the sediment.

The average number of bacteria in the mixed, or whole, milk was 14,388,000; which is slightly less than the average number in the centrifugalized sediment of the same milk.

TABLE 2.

RELATIVE NUMBER OF BACTERIA IN GRAVITY CREAM, CENTRIFUGAL CREAM, AND IN THE SEDIMENT LAYER.

SAMPLE	GRAVITY		CENTRIFUGALIZED		WHOLE MILK
	Cream Layer	Sediment Layer	Cream Layer	Sediment Layer	
11A*..............	2,800,000	35,000	2,200,000	1,100,000	300,000
11B*..............	20,000,000	28,000	9,500,000	640,000	150,000
11C*..............	40,200,000	5,700,000	56,400,000	4,860,000	7,400,000
12A*..............	72,000,000	10,800,000	106,000,000	8,500,000	16,500,000
12B*..............	88,800,000	1,040,000	17,100,000	1,600,000	9,360,000
12C*..............	702,000,000	50,400,000	248,400,000	151,200,000	134,400,000
13A*..............	3,700,000	230,000	17,500,000	756,000	700,000
13B*..............	92,400,000	190,000	167,400,000	11,900,000	17,100,000
13C*..............	50,400,000	630,000	70,600,000	3,300,000	4,800,000
14A*..............	73,800,000	4,800,000	141,600,000	5,550,000	12,600,000
14B*..............	58,800,000	4,100,000	126,900,000	21,600,000	8,600,000
14C*..............	39,380,000	3,100,000	60,000,000	19,600,000	8,000,000
15A†..............	11,400,000	570,000	35,400,000	2,300,000	2,700,000
15B†..............	25,400,000	2,300,000	36,600,000	13,800,000	4,600,000
15C†..............	297,000,000	13,100,000	529,200,000	60,000,000	46,800,000
16A†..............	82,800,000	1,430,000	480,600,000	9,900,000	38,400,000
16B†..............	1,200,000	150,000	4,700,000	490,000	380,000
16C†..............	54,240,000	20,200,000	263,100,000	162,000,000	46,500,000
17A†..............	400,000	42,000	1,700,000	1,600,000	210,000
17B†..............	23,200,000	2,300,000	64,800,000	3,200,000	4,100,000
17C†..............	25,800,000	920,000	22,000,000	432,000	3,600,000
17D†..............	19,600,000	3,800,000	64,800,000	6,400,000	6,800,000
18A‡..............	440,000	100,000	650,000	220,000	50,000
18B‡..............	210,000	5,000	250,000	60,000	47,000
18C‡..............	350,000	13,000	770,000	71,000	35,000
18D‡..............	406,000	22,000	620,000	45,000	46,500
Average.............	68,690,000	4,840,000	97,690,000	18,840,000	14,388,000

*=Sediment not noted; †=Dirty sediment; ‡=No visible sediment.

The enormous difference sometimes found in the bacterial content of the cream and sediment is shown in Sample 13B, in which the cream contained 486 times as many bacteria as the sediment.

From this series it is plain that centrifugally raised cream has slightly more bacteria than gravity raised cream, and centrifugal sediment about four times as many as gravity sediment. This seems not to be dependent upon the presence of dirt, as is seen from the last four samples which were free from visible sediment.

THE RELATIVE NUMBER OF BACTERIA IN GRAVITY CREAM, SKIM MILK, SEDIMENT, AND THE WHOLE MILK.

In studying the relative number of bacteria in gravity raised cream, sediment, skim milk, and the whole milk six samples of milk were examined. The milk was bought in the open market in pint or quart bottles and examined soon after reaching the laboratory. All showed a distinct cream-line. After planting from the cream and sediment layers, 1 c.c. was taken from the mid portion some distance below the cream-line and diluted. After these samples were taken the bottle of milk was well shaken and the sample of whole milk taken, diluted, and plated.

It will be seen from Table 3 that the average number of bacteria in the whole was 2,708,000, in the cream 15,416,000, in the sediment layer 1,405,000, and in the skim milk 2,050,000. From this it will be seen that there is little difference in the number of bacteria in the whole milk, sediment layer, and skim milk, while the cream layer contained about eight times as many per c.c.

TABLE 3.

THE RELATIVE NUMBER OF BACTERIA IN GRAVITY CREAM, SKIM MILK, SEDIMENT, AND IN THE WHOLE MILK.

Sample	Whole Milk	Cream	Skim Milk	Sediment
19A	4,500,000	42,800,000	1,685,000	2,000,000
20	940,000	7,400,000	665,000	570,000
21	7,700,000	18,800,000	7,200,000	4,200,000
22	870,000	6,700,000	580,000	240,000
23	510,000	2,100,000	255,000	220,000
24	2,000,000	14,700,000	1,920,000	1,200,000
Average	2,708,000	15,416,000	2,050,000	1,405,000

THE RELATIVE NUMBER OF BACTERIA IN CENTRIFUGAL CREAM, THE SKIM MILK, SEDIMENT, AND IN WHOLE MILK.

In studying the relative number of bacteria in the cream, sediment, and skim milk of centrifugalized milk seven samples of market milk were examined. The bottle of milk was thoroughly shaken and, after plates had been made of the whole milk, 200 c.c. of the mixed milk were placed in a centrifuge flask and centrifugalized for one hour. The cream was carefully removed, diluted, and plated. Plates were then made from the sediment layer and the skim milk.

It will be seen from Table 4 that the average number of bacteria in the whole milk was 619,000, in the cream 4,500,000, in the sediment 725,000, and in the skim milk only 119,700 per c.c. There was about the same number of bacteria in the whole milk and in the sediment, while the cream contained about 37 times as many bacteria as the skim milk. The great mass of bacteria under the influence of centrifugalization either went up in the cream or down with the sediment, leaving the skim milk with comparatively few bacteria.

TABLE 4.

THE RELATIVE NUMBER OF BACTERIA IN CENTRIFUGAL CREAM, SKIM MILK, SEDIMENT, AND IN WHOLE MILK.

Sample No.	Cream	Skimmed Milk	Sediment	Whole Milk
25	2,000,000	44,666	90,000	120,000
26	1,300,000	44,300	52,500	100,000
27	600,000	6,500	60,000	120,000
28	18,000,000	210,000	800,000	2,400,000
29	7,800,000	470,000	3,530,000	790,000
30	750,000	40,000	409,000	323,000
31	1,050,000	22,000	140,000	480,000
Average	4,500,000	119,700	725,000	619,000

SUMMARY.

The relative number of bacteria in top milk and bottom milk is of great importance in the modification of milk for infant feeding. Top milk sometimes contains from 10 to 500 times as many bacteria per c.c. as the mixed milk.

The preponderance of bacteria in top milk may explain why infants sometimes do not thrive on modified milk made from top milk.

As infection depends to some extent on the number of organisms introduced into the body, top milk on account of its higher bacterial content may sometimes be more harmful than skim milk.

In 30 samples of bottle milk examined the average number of bacteria in gravity raised cream was 69,211,000 and in the sediment layer 4,360,000 bacteria per c.c.

In 26 samples of milk the average number of bacteria in gravity and centrifugally raised cream, in the sediment layer, and in the mixed milk was:

GRAVITY		CENTRIFUGALIZED		WHOLE MILK
Cream Layer	Sediment Layer	Cream Layer	Sediment Layer	
68,690,000	4,840,000	96,690,000	18,840,000	14,388,000

In six samples of milk the average relative number of bacteria in the gravity cream was 15,416,000, in the skim milk 2,050,000, in the sediment layer 1,405,000, and in the whole milk 2,708,000.

In seven samples of milk the average relative number of bacteria in the centrifugally raised cream was 4,500,000, in the sediment layer 725,000, in the skim milk, 119,700, and in the whole milk 619,000.

One sample of milk contained 500 times as many bacteria per c.c. in the cream as in the bottom milk.

When milk is centrifugalized the great mass of bacteria go up with the cream; a lesser number is carried down in the sediment. The skim milk contains many times less numbers of bacteria per c.c. than the cream or sediment layers.

Centrifugally raised cream contains more bacteria per c.c. than the gravity raised cream from the same milk.